Research Article

Preliminary Studies on Vitamin E Self Emulsifying Drug Delivery System based on *Colocynthis citrullus* Seed Oil

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Received: 25-07-2017; **Revised:** 28-08-2017; **Accepted:** 18-09-2017.

ABSTRACT

Vegetable oils are potential pharmaceutical excipients for improving the solubility, stability and bioavailability of poorly soluble drugs. However, there is need to generate scientific data on the possible effect of geographical location on some of the characteristics of these oils. Vitamin E (Vit E) Suffers incomplete absorption in the gastrointestinal tract (GIT). Therefore, the aim of this work was to potentially improve the biopharmaceutical characteristics of Vit. E via SEDDS approach using oil extract from one of the *Colocynthis citrullus (CC)* seeds sourced from three geographical regions in Nigeria. Oil and Lecithin were extracted from CC seeds and oils respectively sourced from Benue, Enugu and Kaduna states in Nigeria. *In vitro* and LD50 evaluations were appropriately carried out on the two extracts. Vitamin E SEDDS were prepared using oil from Kaduna, polysorbate 80 and vitamin E and evaluated for isotropicity, viscosity and droplet size. Results showed that the oil yield ranged from 41 to 46 %. The specific gravity was between 0.896 – 0.956 gcm^{-3} and the refractive index ranged from 0.0075 – 0.0118. The viscosities of the oils were 290.19, 358.01 and 368.47 cp for melon seeds from Enugu, Kaduna and Benue states respectively. The saponification values of melon oil ranged from 181 – 123 KOH/g. The *in vivo* toxicity studies (LD_{50}) showed that there was no significant modification in the general behaviour of the rats and there was no death recorded after oral administration of 10 -5000 mg/kg of the oil. The yield of lecithin from the Cc seed oil was generally low. About 84-87 % of oil and 10 % of polysorbate 80 were optimal for the solubilisation of 2-4 % of vitamin E. The viscosities and droplet sizes of vitamin E SEDDS ranged from 195 to 197 cp and 24.2 to 72.4 (µm) respectively. Therefore, CC seed oil has exhibited acceptable characteristics that favourably position it as a SEDDS constituent with the potential of improved stability and bioavalability of vitamin E.

Keywords: SEDDS, vitamin E, melon oil, lecithin, lipids, drug delivery.

INTRODUCTION

Citrullus colocynthis (cucurbitaceae) seed popularly referred to as Melon in English and "egwusi" in Igbo language (South Eastern Nigeria), is one of the important oil seed crops widely grown and consumed in Tropical Africa[1]. The average protein and oil content of melon seeds are respectively 26.2 % and 47.3 %, and these values are relatively high compared with corresponding values of some common oil-bearing seeds such as cotton with 20.2 % and 21.2% and groundnut with 23.2% and 44.8%, respectively[1]. Compared to soybean seed, melon seed is relatively cheaper, hence the need to further explore the oil derived from this plant as drug delivery carrier.

Plant and animal sources of lipids have been explored as potential excipients for lipid-based formulations[2-7]. Although, chemical specificity and purity may not be optimal, these lipids may be superior to synthetic forms as regards toxicity and biocompatibility [5, 8]. Homolipids (waxes, fats, oils) and heterolipids (phospholipids) have gained renewed interests as excipients for oral drug delivery of lipophilic drugs. The reasons for such interest include a clearer understanding of the manner in which lipids enhance oral bioavailability and reduce plasma profile variability, better characterization of lipidic excipients, formulation versatility, the choice of different drug delivery systems, improved ability to address the key issues of technology transfer and manufacture scale up[9]. Homolipids are esters of fatty acids with various alcohols. The principal materials of interest for oral delivery are long and medium chain fatty acids linked to a glycerol molecule, known as triacylglycerol.

The heterolipids are mainly the phospholipids. Two main classes of phospholipids occur naturally in quantities sufficient for pharmaceutical applications. These are the phosphoglycerides- lecithin and phosphosphingo lipids. Some phosphosphingo lipids such as ceramide are used mainly in topical dosage forms [9].

Lipid-based formulations are typically reputed to improve the solubility and bioavailability of orally administered drugs [5, 10-13]. For poorly water soluble drug molecules, whose dissolution in water is likely the limiting step to overall oral absorption, the primary role of ingested lipids and their lipolytic products is to impart the drug dissolution step by forming – with bile components – different colloidal particles, which are able to maintain a larger quantity of hydrophobic drugs in solution via micellar solubilization [14]. The primary mechanism of action which leads to improved bioavailability is usually avoidance or partial avoidance of slow and more importantly erratic dissolution process which limits the

bioavailability of hydrophobic drugs contained in conventional solid dosage forms [10].

Self emulsifying oil formulation (SEF) is a drug delivery approach that attempts to address the poor solubility of poorly soluble drugs. It is an isotropic mixture of drug, surfactant/s and oil/s or drug, surfactants, cosolvent/s and oil/s. When mildly agitated *in vitro* or *in vivo* in the gastrointestinal tract with aqueous phase it self-emulsifies to produce oil-in-water or water-in-oil emulsions [2]. The terminology ascribed to the emulsion depends on its droplet size and method of preparation. If the final product contains water, it may most likely be called nanoemulsion, if the nanometric size falls outside 100 nm. This is because the presence of water in the final product during the shelf life may impart kinetic instead of thermodynamic stability status to it [15]. If the final product excludes water but contains a surfactant, cosurfactant (with or without a cosolvent) and oil and has a droplet size that is 50 nm and less, it will be called microemulsion. On the other hand a simple mixture of oil and surfactant will yield a product called Self emulsifying drug delivery system (SEDDS).

Our previous works on SEFs successfully addressed the poor solubility of some drugs using some indigenous oils[2]. Our present investigation further explored the usefulness of Cc in the formulation of another poorly soluble drug, vitamine E. Vitamin E is a fat-soluble vitamin which finds application in the pharmaceutical, food and cosmetic industries. It is a free radical scavenging antioxidant with claimed cardiovascular properties[16]. The incomplete gastrointestinal absorption of vitamin E motivated resort to alternative delivery forms, including, surfactants, emulsions or self-emulsifying formulations, which have been reported to give it a biopharmaceutical face-lift and improved bioavailability [15, 17-20]. Therefore, we thought it important to attempt formulation of vitamin E SEDDS with Cc oil and a nonionic surfactant, polysorbate 80 to further explore and widen the horizon of formulation options for improving its bioavailability.

Its association with a surfactant may transform it into an emulsion with improved properties such as permeation enhancement, improved absorption profile and reproducible bioavailability [21]. Of specific importance is the potential role of SEDDS in averting possible intraluminal and/or chemical degradation of vitamin E [22]. In aqueous phase SEDDS constitute oil droplets surrounded by surfactant layer. Ultimately the surfactant establishes a barrier at the interface between the aqueous phase and the oil core.

The Aim of this present work was to potentially improve the biopharmaceutical/bioavailability status of Vit E via SEDDS technique using one of the *Colocynthis citrullus* (*Cc*) oils sourced from three states in Nigeria.

MATERIALS AND METHODS

Materials

The *Colocynthis citrullus* (CC) seeds were obtained from Kaduna main market, Kaduna state, Owulipa Itanabo Market of Benue state and Uzouwani, Enugu state respectively, all in Nigeria. Iodine trichloride, potassium hydroxide, hydrochloric acid (BDH Chemical, Poole, England), chloroform, sodium thiosulphate and acetic acid (Riedell de Haen, Germany), Polysorbate 80 (Merck KGaA, Darmstadt, Germany). All the chemicals used were of analytical grade and were used as supplied.

Extraction of *Colocynthis citrullus* seed oil

A 300 g quantity of each geographical variety was milled (500# grinder/Fuyu Metal, Linyi Fuyu Metal Products Co., Ltd, China). Oil from the seeds of melon was extracted by continuous extraction in Soxhlet apparatus using n–hexane at 40 °C for 12 h. At the end of the extraction the solvent was evaporated in a rotary evaporator.

Characterization of the oil

The oil was characterized for its physicochemical properties including colour, solubility, viscosity, specific gravity, refractive index, acid value, peroxide value, iodine value, and saponification values using the official method of the American Oil Chemists Society [23].

LD_{50} of *Colocynthis citrullus* oil

The acute toxicity test was studied using a method described by Lorke[22]. Four groups of animals consisting of three rats per group were used. The oil was administered orally to the rats using a syringe at a dose of 10 to 5000 mg/kg body weight. The animals were fed and allowed free access to water and observed for death and behavioral changes.

Extraction of lecithin from oil

About 70 ml of Cc seed oil was transferred into a conical flask and heated to 70 °C using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) assembly. Degumming of the oil was done using 2 % of water and some drops of hydrogen peroxide prior to stirring for 1 h. The lecithin obtained was de-oiled by stirring with acetone.

Characterization of lecithin

The lecithin extract was characterized for colour and solubility. The antioxidant properties and test for lecithin were studied using AOCS (1990) methods [21].

Formulationn of Vit E SEDDS using *Colocynthis citrullus* oil as the oil phase

Vitamin E SEDDS were prepared according to the formula in Table 1. Vitamine E was first introduced into the lipid, followed by polysorbate 80. The mixture was subsequently stirred and stored for 48 h at ambient temperature.

Table 1: Composition of vitamin E SEDDS

Batch	Vitamin E (%w/w)	Cc seed oil % (w/w)	Polysorbate 80 (% w/w)
E1	2.1	87.3	10.6
E2	2.5	87.0	10.5
E3	3.0	86.6	10.4
E4	3.6	86.2	10.2
E5	4.8	84.9	10.3

Evaluation and characterization of SEDDS:

Post formulation visual isotropicity/stability test:

The SEDDS stored in well covered transparent bottles were stored at ambient temperature of 30 °C for 48 h and observed for isotropicity (phase separation and/ drug precipitation).

Droplet size measurement

1mL quantity of the SEDDS was diluted in 100 mL of water and gently shaken for complete dispersion. I mL of the dispersion was introduced into plastic cuvette and the droplet size determined using Zeta sizer (Malvern instruments, Germany) [23].

pH measurement

The pH of the SEDDS was determined using a pH meter (pH ep® Hanna instrument, Padova, Italy).

Viscosity measurement

The viscosities of the formulations were determined using a Universal torsion viscometer (Gallenkamp, England) after diluting the SEDDS with distilled water (5 % v/v).

RESULTS AND DISCUSSION

Properties of Cc oil

The oil yield (Table 2) ranged from 41.67 to 46.3 % and showed no statistical difference ($p < 0.05$). These values (especially those from Benue and Kaduna) are in agreement with earlier findings [24, 25, 26]. The oils were characteristically odourless and yellow in colour. The specific gravity values ranged between 0.896 – 0.956 g/cm^3 while the refractive index was between 0.0075 – 0.0118. The viscosity values were between 290-368.47 cp, and the difference in values among the three batches followed this trend, M1 > M3 > M2, with the oil from Enugu recording the least ($p<0.05$) viscosity. The saponification values of the oils ranged from 123-181 KOH/g, with batch M2 having a lower value ($p < 0.05$) than the other two batches. Essentially the values are within acceptable ranges for vegetable oils [21]. Saponification number is the number of milligrams of potassium hydroxide sufficient to neutralize the free fatty acids arising from the complete hydrolysis of 1g of fat/oil. Thus the oil from Enugu evidently had lower quantity of free fatty acid. The iodine value of Batch M2 oil was significantly ($p < 0.05$) lower than those of M1 and M3. The iodine values were also comparable to that obtained from arachis oil and cotton seed oil [27]. Iodine value determines the degree of unsaturation in oils, and it is the quantity of iodine taken up by 100 g of oil. Higher values indicate higher degree of unsaturation in the fatty acid. By implication the oil from Enugu demonstrated the least degree of unsaturation (double bonds). Unsaturation provides sites for attachment of fatty acids or glycerols during transesterification.

Table 2: Properties of Cc seed oil from three geographical locations

Batches	Oil yield (%)	Viscosity (cp)	Specific gravity	Refractive Index	Saponification value of oil (mg KOH/g)	Iodine value	Odour	Colour
M1	46.00±1.0	368.47±2	0.945±0.004	0.0080±0.0008	180±1.0	129.0±1.0	Odourless	Yellow
M2	41.67±0.5	290.19±3	0.896±0.009	0.0075±0.0005	123±0.6	116.5±0.9	Odourless	Yellow
M3	46.30±0.7	358.01±1.2	0.956±0.008	0.0118±0.0003	181±0.9	129.0±0.9	Odourless	Yellow

MI (Benue), M2 (Enugu), M3 (Kaduna)

A curious observation made in this study was that the oils from Benue and Kaduna shared similar properties than that sourced from Enugu. Enugu is in the Eastern flank of Nigeria with more rainfall than Kaduna in the North western part of Nigeria. Benue located at the middle belt region of Nigeria shares similar soil quality that is suitable for some crops commonly grown in the North and in the Eastern part of Nigeria. Soil type and rainfall may have contributed to the similarities and minor variations. Typically, the results evidently confirm the nutritional acceptability of this indigenous food ingredient, and hence the food-industrial applicability. In addition it also strongly points to their applicative potential in the delivery of poorly soluble drugs.

Toxicity test results in Table 3 confirm absence of death among the Wistar rats used. Therefore, CC oil is safe for nutritional purposes, thus portending a potentially safe pharmaceutical excipient. There was also no significant modification in the general behaviour of the rats. Synthetic oils used in lipid drug formulations are products of chemical and thermal modification of vegetable oils. Thus their toxicity profile is a matter of concern. This is why resort to the use of plant-based oils is attracting more attention.

Table 3: LD_{50} of melon seed oil extract

Groups	Dose (mg/kg)	No. of deaths
1	10	0
2	100	0
3	1000	0
4	1600	0
5	2900	0
6	5000	0

Properties of lecithin from Cc seed oil

The properties of lecithin are shown in Table 4 and reveal that the yield of lecithin was generally low. However, the obtained mean values were close to a previously reported value of 1.39 ± 0.17 % [28]. There was no significant ($p<0.05$) difference in the values recorded for the three batches.

Table 4: Properties of lecithin from Cc oil

Batches	Lecithin yield (%)	Phosphate test*	Solubility test†				
			Acetone	Chloroform	Petroleum ether	Methanol	Water
M1	0.49±0.2	++	++	++	++	+	+
M2	0.55±0.3	+	++	++	++	+	+
M3	0.70±0.18	++	+++	+++	+++	++	+
S	-	-	+++	+++	+++	+++	+++

*+- dull yellow, ++- faint yellow, +++- bright yellow, †+- insoluble, ++- slightly soluble, +++- soluble and M1: CC seed from Benue state, M2: CC seed from Enugu state and M3: CC seed from Kaduna state respectively, S- standard lecithin.

As a constituent of the oil used for SEDDS formulation a synergistic effect with vitamin E is a possibility. The results of the phosphate test for lecithin show that lecithin was really the extracted product as shown in Table 4. The three batches of lecithin extract were soluble in chloroform, acetone, petroleum ether, and methanol and to a minimal extent in water. However, standard lecithin positive control demonstrated more aqueous-solubility than the three batches. This may be attributed to extraction deficiencies.

Properties of vitamin E SEDDS

Isotropicity, Droplet size, Viscocity and pH

Only the batches of SEDDS in Tables 1 were visually isotropic after 48 hours storage. Those that lost stability through phase separation were excluded. Isotropicity test is performed in order to evaluate homogenous miscibility, formulation stability and compatibility between the oil and drug phase. Since SEDDS is oil solution of a mixture of drug containing surfactant, assurance of integrity must be established to avoid phase separation or drug crystallization throughout shelf life. Failure of any batch at this stage leads to outright rejection and exclusion[29]. In our preliminary studies about 10 different formulations were evaluated for isotropicity. It was only five that passed the isotropicity test. Isotropic stability is the outcome of careful choice of optimal concentrations of excipients and their overall molecular interaction. In the present investigation 10 % Tween 80 was optimal for the formation of stable SEDDS preparations.

Table 5: The physicochemical properties of vitamin E SEDDS

Batch	Colour	Isotropicity	Viscosity (cp)	pH	Droplet size (µm ± SD)
E1	White	Stable	195.0±0.5	6.0±0.3	42.0 ± 0.2
E2	White	Stable	196.0±0.9	6.0±0.3	24.2 ± 0.1
E3	White	Stable	197.0±0.4	6.0±0.2	72.4 ± 0.2
E4	White	Stable	196.0±0.5	6.0±0.4	36.5 ± 0.1
E5	White	Stable	195.0±0.6	6.0±0.4	48.0 ± 0.3

The viscosity and droplet size ranged from 195 to 197 cp and 24 to 72 µm respectively (Table 5). Generally, there was no observed consistent trend in the above properties.

High viscocity difference between SEDDS and the aqueous phase will cause delayed emulsification in the aqueous environment of the GIT. Also, high viscosity may constitute a positive barrier to drug precipitation during storage. During solidification of liquid SEDDS the quantity of solidifying excipient is reduced by previously increasing the viscosity of the SEDDS.

The pH of the SEDDS recorded values that may not affect its emulsification behavior in the aqueous GIT fluid. The discriminatory role of droplet size is most crucial when gastrointestinal tract (GIT) emulsion stability, biopharmaceutical enhancement and bioavailability improvement are intended. SEDDS may be predisposed to drug precipitation and Oswald ripening after aqueous dilution. Such events as phase separation or droplet growth may adversely alter the droplet size and hasten drug precipitation. Drug precipitation from droplets defeats the concept and goal of SEDDS. Sometimes inadvertent extension of gastric residence time may constitute an inimical platform to promote drug precipitation. However, in our present work, since Vit E is a liquid and not a solid drug, the fear of precipitation is excluded. Thus, the use of liquid drugs in SEDDS is a preferred option because the solubility-related phase transition processes associated with solid drugs are avoided. Furthermore, whereas solid drugs would require careful selection of oils with high capacity to dissolve them, in the formulation of vitamin E SEDDS this tedious exercise is highly reduced to choice of miscible surfactant and oil.

Although our SEDDS have droplet size within micrometer range, they should not be confused with conventional emulsions which require the application of shear force for the dispersed oil phase to form droplets throughout the continuous aqueous phase. SEDDS are fundamentally achieved through simple mixing of oil, surfactant and drug.

Typically, our present SEDDS have better advantage over solid microspheres in that they involve dissolved drug while microspheres are drug crystals entrapped in polymeric matrix.

The high concentration of Cc oil in our formulations is desirable in SEDDS. This is because where as sufficient quantity of surfactant is often a criterion for the formation of stable SEDDS, higher surfactant quantity relative to oil may raise safety concerns. In addition the high oil fraction may involve increased concentration of lecithin which may have a boosting effect on the surface activity of Tween 80. Lecithin has a surfactant property, with a hydrophilic head and lipophyllic tail [31]. Its surfactant property may have acted in synergy with Tween 80 to offer higher stability status to the formulation. Therefore, a stable SEDDS achieved with higher oil than surfactant content will show no potential gastrointestinal toxicity or irritation. However, higher oil content will predispose to higher droplet size. In our previous study melon oil-based indomethacin SNEDDS containing, high (80 % w/w) surfactant concentration recorded droplet size of 195 nm [30].

CONCLUSION

CC seed oil and lecithin were successfully extracted from three batches of melon seeds from Benue, Enugu and Kaduna states in Nigeria respectively. The potential utility of this oil as a lipid excipient for vitamin E SEDDS formulation was investigated. From the overall results we conclude that the oils from Benue and Kaduna states shared similar characteristics. The oils were non-toxic and suitable for the formulation of stable vitamin E SEDDS. Higher oil to surfactant ratio was optimal to form an isotropic vitamin E SEDDS with potential biopharmaceutical enhanced properties. Therefore vitamin E, a necessary vitamin can be formulated into SEDDS for possible improved gastrointestinal absorption.

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Source of Support: Nil, **Conflict of Interest:** None.